

AMENDMENTS TO THE CLAIMS

The following is the status of the claims of the above-captioned application, as amended.

1. (Withdrawn) A method for producing non-2 μ m-family plasmid protein comprising:

- (a) providing a host cell comprising a 2 μ m-family plasmid, the plasmid comprising a gene encoding protein comprising the sequence of a chaperone protein and a gene encoding a non-2 μ m-family plasmid protein;
- (b) culturing the host cell in a culture medium under conditions that allow the co-expression of the gene encoding protein comprising the sequence of the chaperone protein and the gene encoding a non-2 μ m-family plasmid protein; and
- (c) purifying the thus expressed non-2 μ m-family plasmid protein from the cultured host cell or the culture medium.

2. (Withdrawn) The method of Claim 1 further comprising the step of formulating the purified non-2 μ m-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

3. (Withdrawn) A method of producing a non-2 μ m-family plasmid protein comprising:

- a) providing a host cell comprising a 2 μ m-family plasmid, the plasmid comprising a gene encoding protein comprising the sequence of a chaperone protein and a gene encoding a non-2 μ m-family plasmid protein; and
- b) culturing the host cell in a culture medium under conditions that allow the co-expression of the gene encoding protein comprising

the sequence of the chaperone protein and the gene encoding a non-2 μ m-family plasmid protein.

wherein the non-2 μ m-family is a fungal (preferably yeast) or vertebrate non-2 μ m-family plasmid protein.

4. (Currently amended) A 2 μ m-family plasmid comprising a gene encoding peptide disulphide isomerase (PDI) and a gene encoding a non-2 μ m-family plasmid protein.

5. (Withdrawn) A method according to claim 1 wherein the chaperone has a sequence of a fungal chaperone (preferably a yeast chaperone) or a mammalian chaperone (preferably a human chaperone).

6. (Withdrawn) A method according to claim 1 wherein the chaperone comprises the sequence of a protein encoded by any one of *AHA1*, *CCT2*, *CCT3*, *CCT4*, *CCT5*, *CCT6*, *CCT7*, *CCT8*, *CNS1*, *CPR3*, *CPR6*, *EPS1*, *EPO1*, *EUG1*, *FMO1*, *HCH1*, *HSPIO*, *HSP12*, *HSP104*, *HSP26*, *HSP30*, *HSP42*, *HSP60*, *HSP78*, *HSP82*, *JEMI*, *MDJI*, *MDJ2*, *MPD1*, *MPD2*, *PDI1*, *PFD1*, *ABCI*, *APJ1*, *ATP11*, *ATPI2*, *BIT1*, *CDC37*, *CPR7*, *HSC82*, *KAR2*, *LHS1*, *MGE1*, *MRS11*, *NOB1*, *ECM10*, *SSA1*, *SSA2*, *SSA3*, *SSA4*, *SSC1*, *SSE2*, *SIL1*, *SLS1*, *UBI4*, *ORM1*, *ORM2*, *PER1*, *PTC2*, *PSE1* and *HAC1* or truncated intronless *HAC1*.

7. (Withdrawn) A method according to claim 1 wherein the chaperone is protein disulphide isomerase, or comprises the sequence of a protein encoded by *PSE1*, *ORM2* or *SSA1* or a variant or fragment thereof.

8. (Withdrawn) A method according to Claim 1 wherein the host cell also expresses a second recombinant gene encoding a chaperone that is different to the first chaperone encoded by the plasmid.

9. (Withdrawn) A method according to Claim 8 wherein the second recombinant gene encoding a chaperone is chromosomally integrated.

10. (Withdrawn) A method according to Claim 1 wherein the plasmid comprises two different genes encoding different chaperones, one of which gene is the second recombinant gene encoding a chaperone as defined by Claim 8.
11. (Withdrawn) A method according to Claim 8 wherein one of the chaperones is protein disulphide isomerase.
12. (Withdrawn) A method according to Claim 8 wherein one of the-chaperones is ORM2.
13. (Withdrawn) A method according to Claim 8 wherein the two chaperones are protein disulphide isomerase and ORM2.
14. (Withdrawn) A method according to claim 1 wherein the non-2 μ m-family plasmid protein comprises a leader sequence effective to cause secretion in yeast.
15. (Withdrawn) A method according to claim 1 wherein the non-2 μ m-family plasmid protein is a eukaryotic protein, or a fragment or variant thereof, preferably a vertebrate or a fungal (such as a yeast) protein.
16. (Withdrawn) A method according to claim 1 wherein the non-2 μ m-family plasmid protein is a commercially useful protein.
17. (Withdrawn) A method according to claim 1 wherein the non-2 μ m-family plasmid protein comprises a sequence selected from albumin, a monoclonal antibody, an etoposide, a serum protein (such as a blood clotting factor), antistasin, a tick anticoagulant peptide, transferrin, lactoferrin, endostatin, angiostatin, collagens, immunoglobulins, or Immunoglobulin-based molecules or fragment of either (e.g. a dAb, Fab' fragments, F (ab')₂, scAb, scFv or scFv fragment), a Kunitz domain protein interferons, interleukins, IL 10, IL 11, IL2, interferon α species and sub-species, interferon β species and sub-species interferon γ species and sub-species, leptin, CNTF, CNTF_{Ax15} (Axokine™), ILI-receptor antagonist, erythropoietin (EPO) and EPO mimics, thrombopoietin (TPO) and TPO mimics, prosaptide, cyanovirin-N, 5-helix, T20 peptide, T1249 peptide, HIV gp41, HIV gp120, urokinase, prourokinase, tPA, hirudin, platelet

derived growth factor, parathyroid hormone, proinsulin, insulin, glucagon, glucagon-like peptides, insulin-like growth factor, calcitonin, growth hormone, transforming growth factor β , tumour necrosis factor, G-CSF, GM-CSF, M-CSF, FGF, coagulation factors in both pre and active forms, including but not limited to plasminogen, fibrinogen, thrombin, pre-thrombin, pro-thrombin, von Willebrand's factor, α -antitrypsin, plasminogen activators, Factor VII, Factor VIII, Factor IX, Factor X and Factor XIII, nerve growth factor, LACI, platelet-derived endothelial cell growth factor (PD-ECGF), glucose oxidase, serum cholinesterase, aprotinin, amyloid precursor protein, inter-alpha trypsin inhibitor, antithrombin III, apolipoprotein species, Protein C, Protein S, or a variant or fragment of any of the above.

18. (Withdrawn) A method according to claim 1 wherein the non-2 μ m-family plasmid protein comprises the sequence of albumin or a variant or fragment thereof.

19. (Withdrawn) A method according to claim 1 wherein the non-2 μ m-family plasmid protein comprises the sequence of a transferrin family member, preferably transferrin or lactoferrin, or a variant or fragment thereof.

20. (Withdrawn) A method according to claim 1 wherein the non-2 μ m-family plasmid protein comprises a fusion protein, such as a fusion protein of albumin or a transferrin family member or a variant or fragment of either, fused directly or indirectly to the sequence of another protein.

21. (Previously presented) A host cell comprising a plasmid as defined by claim 4.

22. (Currently amended) A host cell according to Claim 21 wherein the gene encoding peptide disulphide isomerase~~a chaperone encoded by the plasmid~~ is an essential gene.

23. (Currently amended) A host cell according to Claim 22 wherein, in the absence of the plasmid, the host cell does not produce the peptide disulphide isomerase~~a chaperone~~.

24. (Previously presented) A host cell according to Claim 21, wherein the host cell is a yeast cell.

25. (Currently amended) A host cell according to Claim 24 in which the 2 μ m family plasmid is ~~based on~~ pSRI, pSB3 or pSB4 and the yeast cell is *Zygosaccharomyces rouxii*, the 2 μ m family plasmid is ~~based on~~ pSB 1 or pSB2 and the yeast cell is *Zygosaccharomyces bailli*, the 2 μ m family plasmid is ~~based on~~ pSM1 and the yeast cell is *Zygosaccharomyces fermentati*, the 2 μ m family plasmid is ~~based on~~ pKDI and the yeast cell is *Kluyveijomyces drosophilum*, the 2 μ m family plasmid is ~~based on~~ pPMI and the yeast cell is *Pichia menabranaefaciens*, or the 2 μ m family plasmid is ~~based on~~ the 2 μ m plasmid and the yeast cell is *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*.

26. (Currently amended) A host cell according to Claim 25 in which the 2 μ m family plasmid is ~~based on~~ the 2 μ m plasmid and the yeast cell is *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*.

27. (Withdrawn) A method according to Claim 1 wherein the host cell is a host cell as defined by Claim 21.

28. (Withdrawn) A method according to Claim 27 wherein the host cell is a host cell as defined by Claim 23.

29. (Withdrawn) A method according to Claim 27 wherein the step (b) involves culturing the host cell in non-selective media, such as a rich media.

30. (Withdrawn) A method for producing non-2 μ m-family plasmid protein comprising:

- (a) providing a host cell comprising a first recombinant gene encoding a protein comprising the sequence of a first chaperone protein, a second recombinant gene encoding a protein comprising the sequence of a second chaperone protein and a third recombinant gene encoding a non-2 μ m-family plasmid protein, wherein the first and second chaperones are

different;

- (b) culturing the host cell in a culture medium under conditions that allow the expression of the first, second and third genes; and
- (c) optionally purifying the thus expressed non-2 μ m-family plasmid protein from the cultured host cell or the culture medium; and .
- (d) optionally, lyophilizing the thus purified protein.

31. (Withdrawn) The method of Claim 30 further comprising the step of formulating the purified non-2 μ m-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

32. (Withdrawn) A method according to Claim 30 wherein the first and second chaperones comprise the sequence of a protein encoded by any one of *AHA1*, *CCT2*, *CCT3*, *CCT4*, *CCT5*, *CCT6*, *CCT7*, *CCT8*, *CNS1*, *CPR3*, *CPR6*, *EPS1*, *EPO1*, *EUG1*, *FMO1*, *HCH1*, *HSPIO*, *HSP12*, *HSP104*, *HSP26*, *HSP30*, *HSP42*, *HSP60*, *HSP78*, *HSP82*, *JEMI*, *MDJI*, *MDJ2*, *MPD1*, *MPD2*, *PDI1*, *PFD1*, *ABCI*, *APJ1*, *ATP11*, *A TPI2*, *BIT1*, *CDC37*, *CPR7*, *HSC82*, *KAR2*, *LHS1*, *MGE1*, *MRS11*, *NOB1*, *ECM10*, *SSA1*, *SSA2*, *SSA3*, *SSA4*, *SSC1*, *SSE2*, *SIL1*, *SLS1*, *UBI4*, *ORM1*, *ORM2*, *PER1*, *PTC2*, *PSE1* and *HAC1* or truncated intronless *HAC1*.

33. (Withdrawn) A method according to Claim 30 wherein the first chaperone is protein disulphide isomerase.

34. (Withdrawn) A method according to Claim 30 wherein the second chaperone is *ORM2*.

35. (Withdrawn) A method according Claim 30 wherein at least one of the first or second chaperones is encoded by a chromosomally integrated recombinant gene.

36. (Withdrawn) A method according Claim 30 wherein at least one of the first or second chaperones is encoded by a gene on a plasmid.

37. (Withdrawn) A method according to Claim 36 wherein the plasmid is a plasmid as defined by Claim 4.

38. (Currently amended) ~~A~~ An isolated host cell ~~of a parent cell~~ comprising a first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) and a second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein, wherein the recombinant gene encoding PDI is on a plasmid ~~and/or the recombinant gene encoding PDI is chromosomally integrated such that the total level of PDI protein production in the host cell is increased relative to the level of PDI protein production in the parent.~~

39. (Canceled)

40. (Currently amended) A host cell according to Claim 38 wherein the transferrin-based protein comprises ~~the sequence of transferrin~~ SEQ ID NO: 80 ~~or any other member of the transferrin family (e.g. lactoferrin), a variant or fragment thereof or a fusion protein comprising transferrin, a variant or fragment thereof~~ or functional fragment thereof.

41. (Previously presented) A host cell according to Claim 38 wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) is provided on a plasmid.

42. (Previously presented) A host cell according to Claim 41 wherein the plasmid is a 2µm-family plasmid.

43. (Previously presented) A host cell according to Claim 38 wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) is chromosomally integrated.

44. (Currently amended) A host cell according to Claim 43 wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) is chromosomally integrated at the locus of an endogenously encoded PDI gene, preferably without disrupting the expression of the endogenous PDI gene.

45. (Previously presented) A host cell according to Claim 38 wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is provided on a plasmid.

46. (Previously presented) A host cell according to Claim 45 wherein the plasmid is a 2 μ m-family plasmid.

47. (Previously presented) A host cell according to Claim 38 wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is chromosomally integrated.

48. (Currently amended) A host cell according to Claim 47 wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is chromosomally integrated at the locus of an endogenously encoded PDI gene, preferably without disrupting the expression of the endogenous PDI gene.

49. (Withdrawn) A method for producing non-2 μ m-family plasmid protein comprising:

(a) providing a host cell comprising a first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof and a second recombinant gene encoding a non-2 μ m-family plasmid protein; and

(b) culturing the host cell in a culture medium under conditions that allow the expression of the first and second genes.

50. (Withdrawn) The method of Claim 49 further comprising the step of formulating the purified non-2 μ m-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

51. (Withdrawn) A method according to Claim 49 wherein the first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof is integrated into the chromosome of the host cell.

52. (Withdrawn) A method according to Claim 49 wherein the first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof is located on a plasmid.

53. (Withdrawn) A host cell comprising first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof and a second recombinant gene encoding a non-2 μ m-family plasmid protein.

54. (Canceled)

55. (Withdrawn) A plasmid comprising a first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof and a second recombinant gene encoding a non-2 μ m-family plasmid protein.

56. (Withdrawn) A plasmid according to Claim 55 which is a 2 μ m-family plasmid.

57. (Withdrawn) A method according to Claim 49 wherein the non-2 μ m-family plasmid protein is as defined in Claim 14.

58-63. (Canceled)

64. (Withdrawn) A method for producing a non-2 μ m-family plasmid protein comprising the steps of:

(a) providing a host cell as defined by Claim 58; and

(b) culturing the host cell in a culture medium under conditions that allow the expression of the essential chaperone and the non-2 μ m-family plasmid protein.

65. (Withdrawn) The method of Claim 64 wherein the host cell comprises a plasmid as defined by Claim 61.

66. (Withdrawn) The method of Claim 64 wherein step (b) is performed by culturing the host cell in a non-selective medium, such as a rich or complex medium.

67-68. (canceled)

69. (Withdrawn) A method for increasing the expression of a non-2 μ m-family plasmid protein in a host cell comprising
providing a polynucleotide comprising a promoter operably connected to a coding sequence encoding a chaperone; and
expressing of the polynucleotide sequence within the host cell,
wherein the promoter is characterized in that it achieves a lower level of expression of the chaperone than would be achieved if the coding sequence were to be operably connected to its naturally occurring promoter.

70. (Withdrawn) A method for producing a non-2 μ m-family plasmid protein comprising the steps of:

(a) providing a host cell comprising a recombinant gene that the sequence of a promoter operably connected to a coding sequence encoding a chaperone, the promoter being characterized in that it achieves a lower level of expression of the chaperone than would be achieved if the coding sequence were to be operably connected to its naturally occurring promoter, and the host cell further comprising a recombinant gene encoding a non-2 μ m-family plasmid protein;

(b) culturing the host cell ~~in a~~ under conditions that allow the expression of the chaperone and the non-2 μ m-family plasmid protein.

71. (Withdrawn) The method of Claim 1 further comprising the step of lyophilizing the thus purified protein.

72. (Withdrawn) The method of Claim 49 further comprising the step of purifying the thus expressed non-2 μ m-family plasmid protein from the cultured host cell or the culture medium.

73 (Withdrawn) The method of Claim 72 further comprising the step of lyophilizing

the thus purified protein.

74. (Withdrawn) The method of Claim 72 further comprising the step of formulating the purified non-2 μ m-family plasmid protein with a carrier or diluent.

75. (Withdrawn) The method of Claim 74 further comprising the step of presenting the thus formulated protein in a unit dosage form.

76. (Previously presented) A host cell comprising a first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) and a second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein, wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase is provided on a plasmid.

77. (Previously presented) A host cell according to Claim 76, wherein the plasmid is a 2 μ m-family plasmid.

78. (Previously presented) A host cell according to Claim 76 wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is chromosomally integrated.

79. (Previously presented) A host cell according to Claim 76 wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is chromosomally integrated at the locus of an endogenously encoded PDI gene, without disrupting the expression of the endogenous PDI gene.

80. (Currently amended) A host cell in accordance with Claim 76 wherein the transferrin-based protein comprises a sequence of transferrin or ~~any other member of the transferrin family (e.g. lactoferrin), a variant or fragment thereof or a fusion protein comprising transferrin, a variant or fragment thereof~~ functional fragment thereof.

81. (Currently amended) A An isolated host cell comprising a first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase and a second recombinant gene encoding a protein comprising the sequence of a transferrin-

based protein wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is chromosomally integrated.

82. (Previously presented) A host cell in accordance with claim 81, wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is chromosomally integrated at the locus of an endogenously encoded PDI gene without disrupting the expression of the endogenous PDI gene.

83. (New) An isolated host cell comprising a first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) and a second recombinant gene encoding a protein having at least 90% sequence identity to SEQ ID NO:80, wherein the recombinant gene encoding PDI is on a plasmid or the recombinant gene encoding PDI is chromosomally integrated, and wherein the total level of PDI protein production in the host cell is increased relative to an otherwise isogenic cell.

84. (New) The host cell of claim 92, wherein the second recombinant gene encodes a protein having at least 95% sequence identity to SEQ ID NO:80.

85. (New) The host cell of claim 92, wherein the second recombinant gene encodes a protein having at least 99% sequence identity to SEQ ID NO:80.

86. (New) The host cell of claim 92, wherein the second recombinant gene encodes a protein having at least 99.5% sequence identity to SEQ ID NO:80.

87. (New) An isolated host cell comprising a first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) and a second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein, wherein the recombinant gene encoding PDI is on a plasmid and the recombinant gene encoding PDI is chromosomally integrated.

88. (New) A host cell according to Claim 43 wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) is chromosomally integrated at the locus of an endogenously encoded PDI gene.

89. (New) A host cell according to Claim 47 wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is chromosomally integrated at the locus of an endogenously encoded PDI gene.